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Chitosan Extraction and its characterization from Black Ant Camponotus compressus (Fab.,) (Hymenoptera: Formicidae

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Abstract: Chitosan has attracted much attention because of its unique biological, chemical and physical properties. Chitosan serves as a promising biopolymer with a variety of potential applications in several industries. Insects are been recommend as a natural resource for chitosan production in several literatures. In the present study, Chitosan has been isolated from adult *Camponotus compressus* by applying standard method. Chitin and chitosan from ants compared with those extracted from shrimps, and were found to be similar. The result shows that ants and shrimp chitosan had the same degree of acetylating and degree of deacetylation of shrimp and ant chitosan was 108.1% and 80.5% respectively, following Fourier transform infrared spectroscopy. The characteristic XRD strong/sharp peaks at 9.4 and 19.4° for α-chitin are common for both ant and shrimp chitin. The percentage ash content of chitosan extracted from *C. compressus* was 1%, which is lower than that obtained from shrimp products. Therefore, chitosan can be obtained in a better quality and of purer form than commercially produced chitosan from shrimp. Therefore, *C. compressus* is a promising alternative source of chitin and chitosan.

Index Terms - Camponotus compressus, **Chitosan, Characterizations of Chitosan.**

I. Introduction

Chitosan are drawing in extraordinary intrigue due to their valuable organic properties, for example, biodegradability, biocompatibility, non-antigenicity and non-lethality (Shahidi and Abuzaytoun 2005). Since they are adaptable biopolymers, their potential applications in different modern fields are as a rule effectively researched. For instance, chitin and chitosan have been reported to be helpful as antimicrobial, emulsifying, thickening and settling specialists in the nourishment business (Shahidi et al. 1999). They have additionally indicated remarkable bioactivity in biomedical fields, including wound mending advancement, resistant framework upgrade, and haemostatic, hypolipidemic and antimicrobial activity.

Insects are the most prevailing species on earth. Insects play numerous important roles for ecosystem services in nature, certain insects provide sources of commercially important products which can be of direct benefit to humans. Also it has become a source for chitosan with unique properties useful for a variety of applications such as an attractive route to the cosmetics, pharmaceuticals, food additives and production of this polymer and body creams, shampoo, lowering of serum and material for production of contact lenses, or eye microbial chitosan as an alternative to the shellfish bandages, permeability control agent, as chromatographic derived product, antimicrobial compounds, seed. Compared to shrimps or crabs (Rodde *et al.* 2008; Youn *et al.* 2009), the cuticle of the insects is much easier to extract chitosan because it contains smaller amounts of crude protein, crude fat, and ash. In this paper, we describe the results of the experiments aimed at optimising the extraction of chitosan from *Camponotus compressus*.

II. Methodology

Adult ants irrespective of sex were procured from vegetable gardens, grasslands and from the local markets in Coimbatore district and used for extraction of chitosan. They were starved for 48hrs before sacrificing and sacrificed ants were dried in oven at 65° + 1C for 48 hrs. After drying the ants were powdered and chitosan was extracted as per the standard method (Burrows *et al.* 2007).

10 gram of ant powder was treated with 4% of NaOH for 1hr in order to dissolve protein and sugar to isolate crude chitin. Samples were boiled in 4% NaOH on hot plate and then it allowed for 30 minutes cooling at room temperature. After cooling each sample was washed thoroughly with deionized water thrice. Supernatant was discarded and residue was demineralised by treating it with 20 ml of 1% HCl for 24 hrs. The demineralized samples were then washed with deionized water. After washing the residue sample were treated with 50 ml of 2% NaOH solution for 1 hr. It decomposes albumin into water soluble amino acids. The supernatant was discarded and remaining chitin was washed with deionized water. The chitin was converted into chitosan by process of deacetylation. The deacetylation process was carried out by adding 50 ml of 50% NaOH to all samples and then boiled at 100°C for 2hours on hot plate. Samples were then cooled for 30 min at room temperature. After cooling Sample were washed continuously with 50 % of NaOH and filtered in order to retain the solid mater. This solid mater is further washed thrice with deionized water. This solid mater was dried in oven at 120° C for 24hrs.

The process of deacylation of chitin to chitosan was confirmed using test as suggested by Kumar and Verma (2012) 5ml of I2 / KI solution was added to each test tube which gives yellow colour to solution. To this solution concentrated sulphuric acid was added , if colour changes from yellow/ brown to dark purple indicate presence of chitosan.

2.1 CHARACTERIZATION OF PREPARED CHITOSAN

The yield of chitosan was obtained by comparing the weight of the raw material to the weight of chitosan, which was obtained after the treatment, while the moisture, colour and appearance were determined according to the AOAC (1990) methods.

Molecular weight

Average molecular weight of chitosan is determined by determination of its intrinsic viscosity by Brook-field viscometer.

pH

The pH measurements of the chitosan solutions will be carried out using a microprocessor pH meter.

Ash value

The ash value of chitosan was determined by 2.0g of chitosan sample placed into previously ignited, cooled, and tarred crucible. The samples are heated in a muffle furnace preheated to 650oC for 4 hr. The crucibles are allowed to cool in the furnace to less than 200ºC and then placed into desiccators with a vented top. Percentage of ash value is calculated using the following.

$$\%\text{Ash} = \frac{\text{Weight of residue (g)}}{\text{Sample weight (g)}} \times 100$$

Loss on drying

Loss on drying of the prepared chitosan will be determined by the gravimetric method.

The water mass loss will be determined by drying the sample to constant weight and measuring the sample after and before drying. The water mass (or weight) will be the difference between the weights of the wet and oven dry samples.

$$\%\text{ loss on drying} = \frac{(\text{Wet weight} - \text{Dry Weight})}{\text{Dry Weight}} \times 100$$

Chitosan Analysis by FT-IR

Fourier transform infrared spectroscopy (FT-IR) was used to determine the presence of the characteristic IR bands, which are the characteristic of chitosan. The FT-IR spectra were taken on an IRTracer-100 spectrometer with a universal Zn-Se ATR (attenuated total reflection) accessory in the 600–4000 cm–1 region. The degree of acetylation (DA) and the degree of deacetylation (DD) of the Ant and shrimp chitosan samples, respectively, were determined by comparing the absorbance of the measured peak to that of the reference peak at A1655/A3450.

X-ray diffraction

XRD analysis was done to evaluate the crystallinity of the ant and the shrimp chitin and chitosan using a D/Max-rA diffractometer. Data was collected at a scan rate of 2° min–1 with the scan angle from 5° to 40°.

Scanning electron microscopy (SEM)

SEM was used for the surface morphology of the chitosan of all the samples to reveal the details of their microstructures. The surface morphologies of the chitin and chitosan were examined with a Quanta 200 FEG ESEM at different magnifications. Before examination, chitosan samples were coated with gold by a sputter coater.

III. Results

Formation of Purple color in test solutions confirmed conversion of chitin to chitosan. The results reviled that chitosan can be successfully obtained from C.compressus.

Table 1: Physical and Chemical Properties of Prepared Chitosan

S.no	Property	Chitosan
1	Color	Pale Yellow
2	Appearance	Powder
3	pH	6.79
4	Degree of Deacetylation (%)	0.89
5	Viscosity(%)	348
6	Ash content %	0.13
7	Un dissolved particles %	0
8	Moisture%	3.33

Chitosan Analysis by FT-IR

The Prolonged decalcification time, even during 24 h, results in a very slight drop in the ash content but can cause polymer degradation the calcium carbonate which dem`onstrates as ash value is very low. The FT-IR studies of the chitosan from standard commercial species. The major absorption band is observed between 1220 and 1020 cm-1 which represents the free amino group (-NH2) at C2 position of glucosamine, a major group present in chitosan. Further the sample showed the absorption bands for the free amino group between 1026 and 1259 cm-1 when the peak at 1374 cm-1 represents the –C-O stretching of primary alcoholic group (-CH2-OH). The absorbance bands of 3268, 2930,2878,1563, and 1418 cm-1 indicated the N-H stretching, Symmetric CH3 stretching and asymmetric CH2 stretching, CH stretching, C=O stretching in secondary amide (amide I) and C–N– stretching in secondary amide (amide II), respectively.

In the present study also the same absorbance bands were observed at 3284, 2931,2866,1653,1558,1427, 1042, 883and 752 cm-1 which confirms the structure of chitosan from table 2 and figure 1.

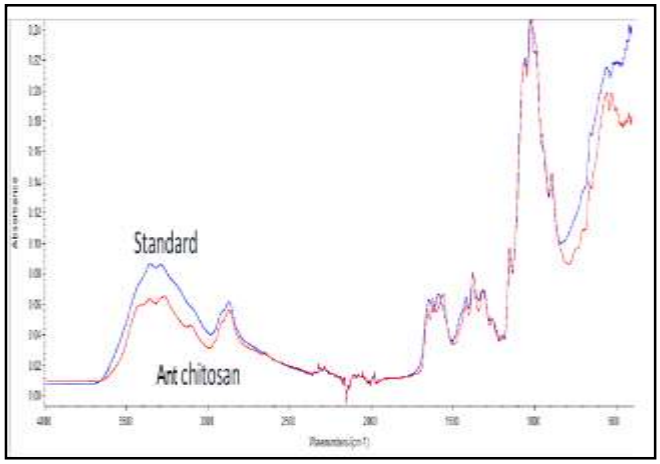

Fig.1 : Comparison of FTIR spectra of standard shrimp chitosan and Ant chitosan.

Table 2: FT-IR band of chitosan isolated from *C. compressus*

Functional group and vibration modes	Classification	Chitosan from *Camponotus compressus*
O–H stretching	------	3439
N–H stretching	------	3103–3257
CH3 symmetrical stretch andCH2 asymmetric stretch	Aliphatic compounds	2881
C–O secondary amide stretch	Amide I	1653
C–O secondary amide stretch	Amide I	1622
N–H bend, C–N stretch	Amide II	1554
CH2 wagging	AmideIII, components of protein	1311
Asymmetric bridge oxygen stretching	---------	1153
Asymmetric in-phase ring stretching Mode	---------	1112
C–O–C asymmetric stretch in phase ring	Saccharide rings	1066
C–O asymmetric stretch in phase ring	--------	1014
CH3 wagging	Along chain	953
CH ring stretching	Saccharide rings	876

The XRD patterns of chitosan

This study also revealed that the chitin extracted from ant in this study is of the α-form and is very similar to that of commercial shrimp chitin. The results of the XRD of chitin extracted from the ant (*C. compressus*) scanned at 2θ, and between 5and 40°, shows a total of ten peaks at 9.4, 12.8, 17.1,19.4, 21.1, 23.2, 26.3, 28.5, 35.0 and 39.0°, while that of commercial shrimp chitin shows a total of nine peaks at 9.4, 12.8, 17.4, 19.4, 21.1, 23.3, 26.2, 28.2 and 39.1°.Both ant and commercial shrimp chitin are similar in that they both have three strong peaks, namely 9.4,19.4 and 21.1°, with 9.4 and 19.4° being the sharpest peaks for both. Also, in the XRD of the ant *C. compressus* and commercial shrimp chitosan (fig 24) , a total of nine peaks were observed for the former, with three strong peaks at 9.6, 19.6 and 21.2°, and six weak ones at 12.4, 23.0, 26.2, 28.5, 35.0 and 39.0°. Commercial shrimp chitosan showed a total of 15 different peaks, with three strong peaks at 10.1, 20.2 and 22.3° with others asweak peaks. It has been revealed in previous studies

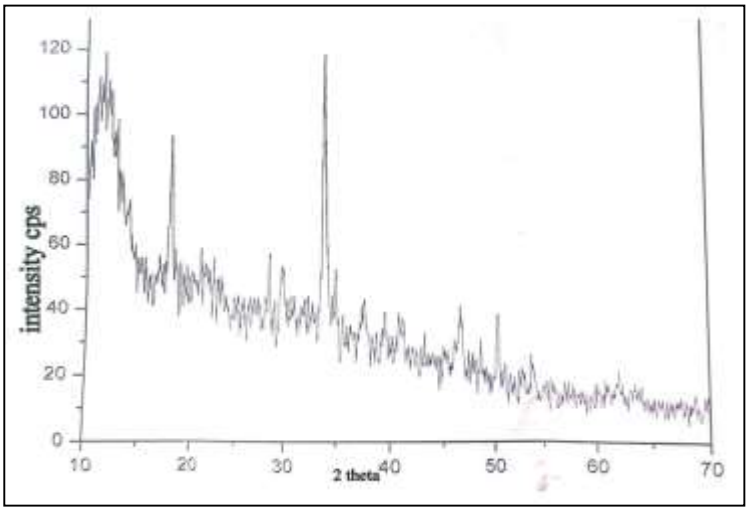

Fig. 2: XRD patterns of chitosan

SEM image of Chitosan

The morphology of chitosan was studied by SEM and FESEM and the micrographs at different magnifications and different area of chitosan are shown in figure 3. From this study, the surface area morphology of house cricket chitin and chitosan is different from those of commercial (shrimp) chitin and chitosan. Generally, at lower magnification (×500) ant chitosan shows a combination of rough and smooth layers of flakes; also big pores can be seen in some areas, while febrile structures can easily be distinguished in other parts (figure 18). At different magnification (×1000), ant chitosan has big pores that are surrounded by numerous nano pores on a smooth surface devoid of fibres.

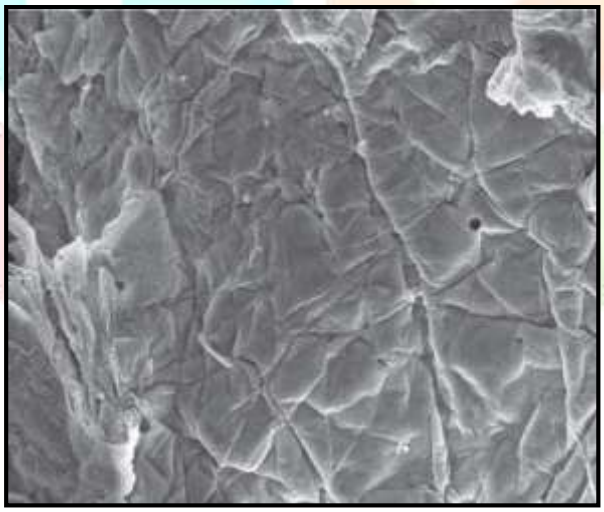

Fig. 3: SEM image of Chitosan

IV. Conclusion

Keeping in view the medicinal uses of ants in traditional remedies chitosan extracted from *Camponotus compressus* can be used in Pharmaceutical, cosmetic and chemical industries. Chitosan was isolated from adult *C. compressus* using standard methods. Insect cuticles have lower levels of inorganic material compared to crustacean shells, which makes their demineralization treatment more convenient. The characteristics of chitosan from adult *C. compressus* were similar to those of commercial chitosan from shrimp by FTIR analysis. Thus *C. compressus* is an alternative source of chitosan. The large numbers of *C. compressus* adults captured for the control of this pest in fields every year provide an abundant source for the production of chitosan. In addition, attempts to domesticate the ants will help relieve the impact to ecological systems in the future.

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